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The role of cyclic AMP in isoprenaline-induced cardiac necroses in the rat*

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Single administrations of isoprenaline chloride (6–36 μg/kg, s.c.) resulted in the appearance of acute myocardial necroses in the rat within 24 h. The percentage of animals with lesions was directly related to the dose of isoprenaline. Dibutyryl cyclic AMP (1–50 mg/kg, i.p.) induced myocardial lesions of the same type, however the percentage of animals affected was not dose-dependent. Pretreatment with theophylline (75 mg/kg, s.c.) administered to inhibit cardiac phosphodiesterase activity, induced a 48-fold reduction in the ED50 of isoprenaline and potentiated the nucleotide-induced cardiac lesions which became dose-dependent. Thus, a mediatory role for cyclic AMP in the isoprenaline-induced cardiac necroses in the rat is suggested.

It is well known that isoprenaline and other catecholamines are capable of inducing myocardial necroses in the rat (Rona, Chappel & others, 1959; Chappel, Rona & others, 1959; Wexler & Kittinger, 1963; Ferrans, Hibbs & others, 1964; Kahn, Rona & Chappel, 1969). β-Adrenergic blocking agents were found to prevent competitively the appearance of these lesions (Mehes, Rajkovits & Papp, 1966; Dorigotti, Gaetani & others, 1969). Since Robison, Butcher & Sutherland (1967) suggested an association of the β-receptors with adenyl cyclase, this could indicate involvement of the adenyl cyclase-cyclic AMP system in the production of these lesions. This possibility was further strengthened by the finding that a rise in the heart levels of cyclic AMP occurred in the rat after catecholamine administration (Robison, Butcher & others, 1965; Namm & Mayer, 1968). Thus, if the cardiac lesions induced by the catecholamine isoprenaline were mediated through cyclic AMP, the phosphodiesterase inhibitor theophylline, by preventing the breakdown of cyclic AMP (Butcher & Sutherland, 1962) should potentiate the isoprenaline-induced lesions. Furthermore, the administration of exogenous cyclic AMP alone or in the presence of theophylline should induce similar pathological changes. This report deals with the results obtained in testing these two concepts.

METHODS

Male Wistar rats in the weight range of 250–270 g were used. The animals were divided into six groups as indicated in Table 1. Group I served as control and was injected with vehicle (normal saline). Groups II, III and V received isoprenaline, theophylline, or dibutyryl cyclic AMP respectively, at various doses. Groups IV and VI were pretreated with theophylline, followed in approximately 20 min by isoprenaline or dibutyryl cyclic AMP, respectively.

Twenty-four h after drug administration all animals were killed by decapitation.

* A preliminary report of some of these results was presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, August 1970, Palo Alto, California.

The hearts were removed, fixed in a solution of 5% formal saline dehydrated in ethanols (70 to 90%) followed by amyl acetate (100%), cleared in toluene and embedded under vacuum in paraffin. Four longitudinal sections (6 μm) of each heart were made and stained with haematoxylin eosin. The hearts were considered lesioned when, under microscopical examination, at least one focus of acute myocardial necrosis was observed (Dorigotti & others, 1969). When applicable, drug ED50's and 95% confidence limits for the production of myocardial necroses were calculated by the methods of Litchfield & Wilcoxon (1949).

All drugs were freshly dissolved in normal saline and injected in volumes of 0·2 ml/100 g subcutaneously, with the exception of dibutyryl cyclic AMP which was injected intraperitoneally. Doses used are expressed as salts, with the exception of theophylline which is expressed as base.

The following drugs were used: theophylline ethylenediamine (aminophylline, BDH); dibutyryl cyclic AMP (N^6–$O^{2'}$-dibutyryl adenosine-3′-5′-cyclic phosphate monosodium,5H_2O, Calbiochem); (±)-isoprenaline hydrochloride [(±)-isopropylnoradrenaline hydrochloride, K & K Labs].

RESULTS

At macroscopic examination, no gross pathological changes were seen in rat hearts of any group 24 h after drug administration.

Histologically, foci of acute myocardial necrosis were seen in the cardiac ventricles of a certain percentage of rats in all drug-treated groups. The areas most frequently affected were the papillary muscles and the apices; the ventricular walls, however, were also sometimes affected. The lesions appeared as typical, acute, focal necroses consisting of fragmentation and vacuolization of the myocardial fibres, and mononuclear inflammatory cell infiltrates with a few scattered polymorphonuclear leucocytes. Organized, chronic cardiac lesions were seen in approximately 2% of rats used and were considered to be endemic in the rat population. In all groups, only those animals showing the acute type of lesions (acute myocardial necroses) were considered in the results.

As indicated in Table 1, no acute myocardial lesions were seen in the saline-treated rats (Group I). After isoprenaline (Group II) the number of animals showing acute focal myocardial necroses was found to be dose-dependent, the ED50 being 13 μg/kg. Theophylline (Group III) caused acute focal cardiac necroses in only 1/10 rats at 75 mg/kg and in 3/10 rats at 150 mg/kg whereas higher doses were lethal. On the other hand, pretreatment with the lower dose of theophylline (75 mg/kg) resulted in a 48 fold reduction in the ED50 of isoprenaline, it now being 0·27 μg/kg (Group IV).

The occurrence of lesions with dibutyryl cyclic AMP (Group V) were not dose-dependent, 1/10 rats presenting acute focal cardiac necroses at 1 (Fig. 1A), 10 and 50 mg/kg. Once again, however, pretreatment with theophylline (75 mg/kg) markedly facilitated the production of cardiac lesions resulting from the administration of dibutyryl cyclic AMP (Group VI), which now appeared to be dose-dependent, the ED50 was 0·55 mg/kg.

Thus, pretreatment with theophylline markedly increased the number of animals exhibiting acute focal myocardial necroses after administration of either isoprenaline or dibutyryl cyclic AMP. Additionally, in some affected animals the histological picture changed from that of an isolated focus to that of disseminated focal necroses (Fig. 1B).

Table 1. *Number of rats showing acute myocardial necroses.**

Group	Treatment	Dose/kg	Route	No with lesions/ No tested	ED50/kg	95% Confidence Limits
I	Normal saline	—	s.c.	0/30	—	—
II	Isoprenaline	6 μg	s.c.	1/10		
		14 μg	s.c.	6/10	13·00 μg	(8·10–20·80)
		36 μg	s.c.	9/10		
III	Theophylline	75 mg	s.c.	1/10	>150·00 mg	—
		150 mg	s.c.	3/10		
IV	Theophylline† + isoprenaline	0·1 μg	s.c.	4/10		
		0·3 μg	s.c.	5/10	0·27 μg	(0·09–0·81)
		1·0 μg	s.c.	8/10		
V	Dibutyryl cyclic AMP	1 mg	i.p.	1/10		
		10 mg	i.p.	1/10	>50·00 mg	—
		50 mg	i.p.	1/10		
VI	Theophylline† + dibutyryl cyclic AMP	0·25 mg	i.p.	4/10		
		0·50 mg	i.p.	4/10	0·55 mg	(0·23–1·26)
		1·00 mg	i.p.	7/10		

* Hearts were examined 24 h following treatment.
† Theophylline (75 mg/kg, s.c.) was administered 20 min before isoprenaline or dibutyryl cyclic AMP. Doses listed refer to the latter two compounds.

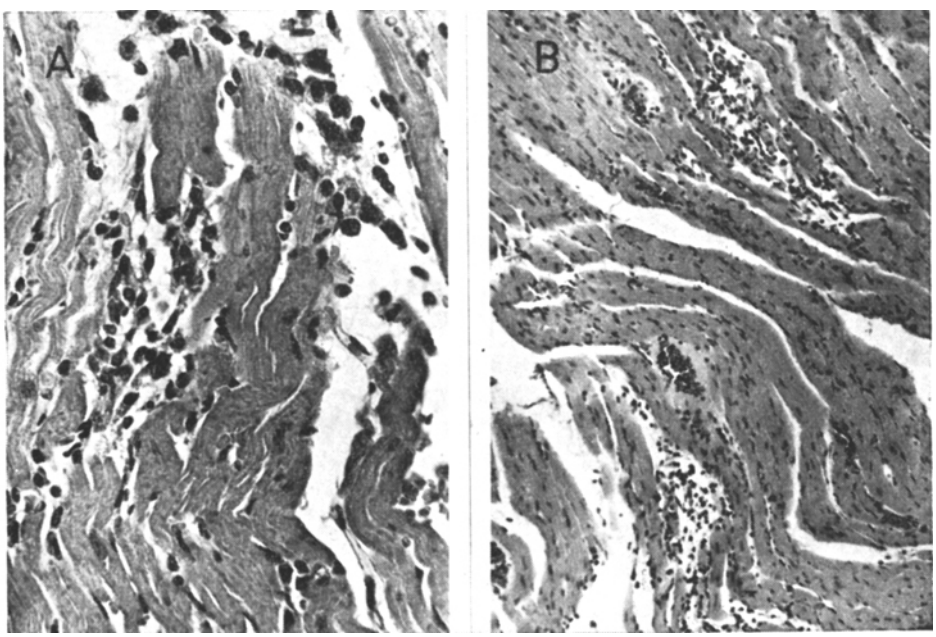

FIG. 1.A. Photomicrograph of a section of a heart of a rat injected with dibutyryl cyclic AMP (1 mg/kg, i.p.) and killed at 24 h. A necrotic focus is shown. H.E. × 630.
B. Photomicrograph of a section of a heart of a rat injected with theophylline ethylenediamine (75 mg/mg, s.c.) and dibutyryl cyclic AMP (1 mg/kg, i.p.) and killed at 24 h. Disseminated foci of necrosis are shown. H.E. × 196.

DISCUSSION

Robison & others (1965) and Namm & Mayer (1968) showed that a rise in the myocardial level of cyclic AMP occurred in the rat after catecholamine administration. This increase in cardiac cyclic AMP levels appearently results from stimulation of β-adrenergic receptors, which are thought to be associated with adenyl cyclase (Robison & others, 1967). Thus, the finding that β-adrenergic blocking agents competitively prevent the appearance of catecholamine-induced myocardial necroses in this species (Mehes, Rajkovits & Papp, 1966; Dorigotti & others, 1969) suggested an involvement of the adenyl cyclase-cyclic AMP system.

In the present experiments, support for the involvement of this system in catecholamine-induced myocardial necroses was provided by the use of theophylline, a phosphodiesterase inhibitor which prevents the breakdown, and thus, inactivation of cyclic AMP (Butcher & Sutherland, 1962). Indeed it was found that even minimal doses of theophylline markedly potentiated isoprenaline, a β-adrenergic receptor stimulant, in its ability to induce acute myocardial necroses.

On the other hand, administration of dibutyryl cyclic AMP alone failed to mimic the isoprenaline-induced lesions on the rat heart. However, Robison & others (1965) also did not succeed in mimicking the myocardial effects of catecholamines in the isolated perfused rat heart with either cyclic AMP or the dibutyryl derivative. Apparently, these authors found that the rat heart exhibits a low permeability toward the nucleotides, coupled with a high phosphodiesterase activity. This latter aspect may be the more important one, since as shown in the present experiments, pretreatment with theophylline markedly potentiated the dibutyryl cyclic AMP-induced acute myocardial lesions, which became dose-dependent. This finding provides additional support for the involvement of cyclic AMP in catecholamine-induced cardiac lesions.

Consequently, in rats a mediatory role for the adenyl cyclase-cyclic AMP system may explain the pathological changes induced in the myocardium by the administration of β-adrenergic receptor stimulants such as isoprenaline.

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